Linking drugs of abuse in wastewater to contamination of surface and drinking water

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Abstract

The concentrations of 17 drugs of abuse (DOAs), including cocaine, several amphetamine, opioid drugs and two metabolites, benzoylecgonine (BE) a metabolite of cocaine and 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) a metabolite of methadone, were investigated in an urban watershed that is heavily impacted by discharges of municipal wastewater. The artificial sweetener, sucralose was also monitored as a persistent tracer of contamination from municipal wastewater. Monitoring was conducted in a municipal wastewater treatment plant (WWTP) and at sites upstream and downstream of the WWTP discharge, as well as in a drinking water treatment plant (DWTP) located 19 km downstream of the WWTP discharge that withdraws raw water from the river. Drug concentrations were monitored with Polar Organic Chemical Integrative Samplers (POCIS) deployed for 2 weeks in the river and in the WWTP and DWTP. Several of the investigated compounds exhibited a decrease in concentration with distance downstream from the wastewater discharge into the river, but there was little attenuation of sucralose, cocaine, BE, morphine, acetylmorphine (a-morphine),

acetylcodeine (a-codeine) and oxycodone. Heroin and methadone were not detected at any sample locations. Amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxy-methamphetamine (MDMA), and EDDP were not detected in the samples collected at the drinking water intake. Many of the DOAs were not removed effectively in the DWTP, including cocaine, BE, methylenedioxyamphetamine (MDA), ephedrine, and several prescription opioids, most probably because the DWTP was operating at or above its rated treatment capacity. These data indicate that there can be transport of DOAs from wastewater sources into drinking water in urban watersheds.

Keywords: Cocaine, amphetamines, opioids, POCIS, drugs of abuse

1 INTRODUCTION

Drugs of abuse (DOAs), including both illicit drugs and prescription drugs are contaminants of emerging concern (CECs) that have been detected in wastewater and surface waters [1]. After excretion as either the parent compound or as metabolites, these classes of drugs make their way to wastewater treatment plants (WWTPs). Many studies have investigated the presence of DOAs in wastewater and it is clear that many of these compounds are not completely removed during conventional wastewater treatment and that they are released into surface waters via effluent discharges [2-9]. DOAs have potent biological activities and they may impact aquatic organisms, either alone or as mixtures with other pharmaceuticals [10, 11]. Pharmaceuticals can be transformed through biological, chemical or photochemical processes or can be adsorbed, depending on the nature of the compound [12, 13]. In areas where surface

waters are used as sources of drinking water, DOAs may also contaminate drinking water [14, 15]. Wastewater reuse is becoming more common and there is concern about the presence of DOAs in water supplies [16].

Since concentrations of CECs in the aquatic environment are subject to large temporal variations, passive sampling has been used to provide estimates of the time weighted average (TWA) concentrations of compounds over several days or weeks of sampler deployment [17]. The Polar Organic Chemical Integrative Sampler (POCIS) was developed by Alvarez et al. [18] to sequester hydrophilic organic chemicals from aquatic matrices, and TWA concentrations can be calculated using sampling rates (R_s in $L\ d^{-1}$) measured experimentally for each target analyte. POCIS have been shown to be effective at sampling pharmaceuticals and pesticides from water [19, 20] but relatively few studies have used POCIS to estimate the concentrations of DOAs in water and wastewater [16, 21-23].

The objective of this study was to evaluate the transport of DOAs from wastewater to drinking water in an urban watershed. A range of DOAs, including cocaine and its primary metabolite (BE), EDDP a metabolite of methadone, amphetamines and opioid compounds were monitored as they were transported through a WWTP, carried downstream in a river from the point of wastewater discharge, and subsequently taken up and treated in a downstream drinking water treatment plant (DWTP). The study area was the Grand River in southern Ontario, Canada, which receives effluents from several municipal WWTPs, and also serves as a source of drinking water for several communities [24, 25]. Our hypothesis is that these compounds are persistent

enough in aquatic systems and may potentially impact the quality of drinking water. To test this hypothesis, we compared the concentrations of DOAs to the concentrations of an artificial sweetener, sucralose that is known to be a persistent tracer of wastewater contamination [26, 27] and has been detected previously at high concentrations in the Grand River [28].

2 MATERIALS AND METHODS

2.1 Chemicals, reagents and supplies

Analytical standards of all DOAs and their deuterated analogs (list of compounds provided in the Supplemental Data, Table S1) were obtained from Cerilliant at a purity higher than 99%. Sucralose and its deuterated surrogate were purchased from Sigma-Aldrich and Toronto Research Chemicals, respectively. Stock solutions were made up in methanol and stored in the refrigerator (4°C) until required. Working standard solutions were prepared from stock solutions through serial dilution.

HPLC or equivalent grades of methanol, acetone, acetonitrile, ACS reagent grade dichloromethane (DCM), hydrochloride acid (37%), sulphuric acid (96%), formic acid (88%), and trace metal grade ammonium hydroxide (88%) and sodium sulfate were purchased from Fisher Scientific. Optima grade methanol, acetonitrile, and water were also purchased from Fisher Scientific. All other water was obtained from a Milli-Q water purification system purchased from Millipore. Whatman 1.5 µm glass microfiber filters were purchased from Fisher Scientific. Oasis MCX cation exchange cartridges (6 mL/150 mg) were purchased from Waters

Corporation. Pharmaceutical POCIS containing Oasis HLB (200 mg) sequestration medium were purchased from Environmental Sampling Technologies.

2.2 Samples

POCIS samplers were deployed over a two week period from July 10-24, 2012 at locations in the Grand River watershed in southern, Ontario, Canada. POCIS monitoring was conducted at a WWTP in the treated wastewater stream, at sites in the Grand River upstream (2 locations) and downstream (3 locations) of the WWTP discharge, and in the raw and treated water streams of a DWTP that uses the river as a source of water. POCIS (n=3 per location) were deployed on July 10 and retrieved two weeks later. Grab samples were collected in 1 L pre-cleaned amber glass bottles on the days of POCIS deployment and retrieval in order to compare the concentrations of target compounds detected using both methods. The WWTP treats wastewater for an urban population of approximately 94,000 using conventional activated sludge treatment and has a maximum flow capacity of 81,800 m³ d⁻¹. Surface water samples were collected and POCIS were deployed in the Grand River at two points approximately 2 km apart located upstream of the WWTP discharge and at three downstream sites approximately 40 m downstream of the discharge, and at 10 km and 18 km further downstream. The furthest downstream site was only 20 m from the intake for the DWTP. The small DWTP serves a population of approximately 1,800 people and the design flow is 1.6 x 10⁶ L d⁻¹. The plant uses a limited treatment train consisting of coagulation, flocculation, filtration and chlorine disinfection. POCIS were deployed in the treatment stream for both raw and treated drinking water.

POCIS samplers were kept in air-tight canisters prior to deployment. A total of 6 POCIS were deployed at each site in a stainless steel sampling cage; three POCIS for monitoring DOAs and three POCIS for monitoring sucralose. POCIS field blanks were exposed to ambient air during the deployment and retrieval of the POCIS samplers. Upon retrieval, each POCIS disk was individually wrapped in aluminum foil and stored in a sealable plastic bag and placed on ice during transportation. Following collection, samples were stored in a cooler at 4°C for transport to the laboratory, where they were then stored at -20°C until extraction, which was carried out within one month of collection.

2.3 Extraction

POCIS samplers were extracted according to methods previously described by Li et al. [29]. Briefly, frozen samplers were removed from storage and allowed to thaw, then rinsed with water to remove debris and biofouling material. The sorbent in the POCIS was transferred manually to a glass chromatography column previously packed to 1/3 full with granular Na₂SO₄. Deuterated analogs were then added to the column as surrogates. Elution from the column was performed with 100 mL methanol. After evaporation, the samples were made up to their final volume (0.4 mL) with methanol. The DOAs and sucralose were sampled in individual POCIS that were extracted separately. Extraction efficiencies of all compounds in the three matrices were >80% (Supplemental Data, Table S1).

Grab samples were extracted in triplicate by solid phase extraction (SPE) with Oasis MCX cartridges using previously developed methods. The DOAs and sucralose were extracted

separately using two different methods. Prior to SPE, all aqueous samples were vacuum filtered through 1.5 μm glass fibre filters. For untreated wastewater samples, 100 mL volumes were filtered, whereas for treated wastewater, surface water, raw and treated drinking water samples, a volume of 200 mL was used. All samples were acidified to a pH of 2.5 using 3.5 M sulphuric acid and then spiked with 100 μL of 500 ng mL^{-1} surrogate standard mixture. For DOAs, SPE was carried out using a Gilson GX-271 ASPECTM automated extraction system. The extraction method was based on our previously published method for illicit drugs [25] that was modified to include opioid drugs [22]. Sucralose was extracted by SPE using the manual SPE method previously described by [30]. Extracts were evaporated and reconstituted to a volume of 0.4 mL in 25% water/75% methanol. SPE recoveries for all compounds in each of the three matrices were >80% (Supplemental Data, Table S1).

2.4 POCIS sampling rates

Sampling rates (R_s) for the most DOA target compounds were previously reported by Yargeau et al. [22] and are listed in the Supplemental Data, Table S1. Note that R_s values for acetylcodeine, acetylmorphine and heroin could not be determined, which is probably due to the highly polar nature of these compounds [22]. The sampling rate for sucralose was previously described by Metcalfe et al. [30]. The POCIS sampling rates for ketamine ($0.197 \pm 0.007 \text{ Ld}^{-1}$) and fentanyl ($0.390 \pm 0.051 \text{ Ld}^{-1}$) are reported here for the first time.

The sampling rates (R_s) were determined as previously described by Li et al. [29] using bench scale experiments with static exposure conditions where the decrease in concentration of the compounds in water was monitored over time. Briefly, the static experiments were

conducted in triplicate in containers with 3 L of water placed in a temperature controlled environmental chamber at 25°C. For each replicate, the water was spiked with the compounds of interest at a nominal concentration of 3 µg L⁻¹ and a single POCIS was placed in the water for a period of 3 days. A magnetic stirrer was used to gently mix the water. Aliquots of the exposure water (40 mL) were removed from the bottles every 24 h to monitor the decrease in water concentration over time. The water was extracted by SPE according to the methods described above. Control experiments containing only fortified water without the POCIS (i.e. positive control) were run along with the calibration to correct for sorption, volatilization or degradation during exposure. As a negative control, one POCIS was exposed to 3 L of water without spiking of pharmaceuticals. The R_s of the POCIS was calculated from the slope of the line describing the decline in concentration over time. As in previous experiments to determine sampling rates with this static exposure method [19, 29], the sampling rates were confirmed by comparing estimates of the mass of the target compounds accumulated on the sorbent over the 3 day experiment to the measured mass of the compound extracted from the POCIS at the end of the experiment. The estimates of the mass accumulated on the POCIS over time, $m_s(t)$ were determined by rearranging the equation presented by Allan et al. [32] to yield:

$$m_s(t) = m_s(0) + C_w R_s t$$

Where: The amount of the analyte on the sorbent at time 0, $m_s(0)$, which was determined from control experiments to be zero, C_w is the initial concentration in the water, R_s is the estimated sampling rate, and $t = 3$ days. These data for fentanyl and ketamine are illustrated in Supplemental Data, Figure S1. The results indicate good agreement between the estimated and measured amounts of the target compounds retained by the POCIS sorbent. Further, these data

indicate that the amounts of the target compounds retained by the POCIS membrane (not extracted) are negligible relative to the amount retained by the solid sorbent.

2.5 Analysis

The concentrations of DOAs were measured by liquid chromatography with high resolution mass spectrometry (LC-HRMS) using an Accela LC system coupled to a hybrid linear ion trap-orbital trap instrument, LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA). Chromatographic separation of the target compounds was achieved using a Hypersil Gold column (50 x 2.1 mm, 1.9 μm) with an in-line Direct-Connection UHPLC 0.2 μm filter held at a temperature of 30 °C. An optimized gradient of methanol in water (solvent A) and acetonitrile (Solvent B) both with 0.1% acetic acid at a flow of 0.3 mLmin⁻¹ was used. The percentage of organic (B) was changed as follows: 0 min (10%), 1.75 min (10%), 3.05min (25%), 5.55 min (97%), 7.55 min (97%), 7.75 min (10%), 16.00 min (10%). Ionization was done in positive mode using a heated electrospray ionization (HESI) source with the following parameters: sheath gas flow = 45 arbitrary units, auxiliary sheath gas flow = 10 arbitrary units, capillary temperature = 375°C, capillary voltage = 5 V, tube lens = 100 V. Acquisition was performed in full scan mode (m/z 50-400) at high resolution ($R_{\text{FWHM}} = 41\,000$) and analyte quantification was carried out by extracting the ion of interest from the orbital trap total ion current chromatogram using an m/z window of ± 0.01 . Confirmation of the presence of the target analyte was done by collision-induced mass spectra using a data dependent tandem mass spectrometry experiment in the linear ion trap portion of the instrument. Quantification was done using an eight-point calibration curve generated for each compound in the range of 3 to 150 $\mu\text{g L}^{-1}$, corresponding to the range of concentration obtained after preconcentration, as well as the linear correlation coefficients. The

deuterated stable isotope surrogates were used as internal standards at a constant concentration of 100 $\mu\text{g L}^{-1}$ to correct for mass losses during sample preparation, as well as matrix effects during analysis.

Analysis of sucralose was conducted by LC coupled with tandem mass spectrometry (LC-MS/MS) in negative ion mode using an AB Sciex Q-Trap 5500 instrument with a turbospray ionization source, and equipped with an Agilent 1100 series (Mississauga, ON, Canada) separation system. Sucralose was separated chromatographically using a Genesis C-18 column and a guard column of the same stationary phase (Chromatographic Specialties, Brockville, ON, Canada). The LC mobile phases for gradient elution were described previously by Metcalfe et al. [30]. Multiple reaction monitoring (MRM) was performed using the precursor and product ion transitions of 395 $\rightarrow$ 359 and 395 $\rightarrow$ 35 for sucralose, and 403 $\rightarrow$ 367 and 403 $\rightarrow$ 35 for the corresponding labelled surrogate, sucralose-d6. An external standard method with a five-point calibration curve was used for quantification, and the data were adjusted according to response for the surrogate internal standard.

2.6 Method validation

The limits of detection (LODs) and limits of quantification (LOQs) for the target compounds in wastewater, surface water and drinking water were defined as the analyte concentration that produced a peak with a signal to noise ratio of 3 and 10, respectively. All method validation was done using spiked samples of each matrix. The LODs and LOQs of the target compounds in each matrix are listed in the Supplemental Data, Table S2. The repeatability between runs on the same day and between days was always <15%. Quality control samples in

each matrix were included in sample runs and the relative error between the measured concentration and the expected concentration was always <20% for all target compounds. Field blank POCIS were processed exactly as described for the deployed POCIS and none of these samples contained residues of the analytes at levels above the limits of detection.

3 RESULTS AND DISCUSSION

3.1 Overview of the results

The concentrations of the analytes in the Grand River at sites upstream and downstream of the WWTP discharge, as well as the concentrations in treated wastewater in the WWTP and in raw and treated drinking water in the DWTP are summarized in Figure 1. These data are the estimated TWA concentrations from the POCIS (n=3). The values of the average concentrations and corresponding standard deviations are provided in the Supplemental Data, Table S3 along with the concentrations measured in grab samples collected at deployment and retrieval. The concentrations measured in grab samples were used only to provide a comparison point to determine if POCIS was an effective monitoring technique, despite the potential for biofouling of the samplers or blockage of the deployment cages. Concentrations of some analytes measured in grab samples were lower than TWA concentrations estimated from POCIS, consistent with the results of a study by Jones-Lepp et al. [16]. In some cases, analytes were detected or quantifiable in POCIS but not in the corresponding grab samples, which illustrates the value of passive sampling for concentrating trace contaminants to detectable levels, and the importance of effective sampling strategies. Overall, the results are in good agreement and no significant variations were observed in the grab samples collected at deployment and retrieval.

Concentration observed in upstream samples can be explained by the presence of upstream WWTPs on discharging their treated effluent in the same river.

Considering that our previous studies showed that POCIS underestimated the concentrations of DOAs in untreated wastewater (Metcalfé et al., 2010), we did not deploy POCIS in the untreated wastewater stream and so, did not attempt to calculate removals of DOAs within the WWTP. However, our previous studies of monitoring for pharmaceuticals in DWTPs using POCIS showed that this monitoring technique provides reliable estimates of time-weighted average concentrations in treated and untreated drinking water that are free of the biases from grab sample monitoring caused by the residence time in the DWTP (Metcalfé et al., 2014). Table 1 presents the average removals (%) of each compound during drinking water treatment. The statistical significance of the values, identified by an asterisk, was determined using the Software Prism 6 and unpaired t-tests. This small DWTP was operating above its designed capacity during the sampling campaign and so therefore, cannot be considered as representative of the drinking water treatment in most developed countries. However, these results indicate that, under conditions of sub-optimal treatment, contamination of surface water with DOAs of wastewater origin can impact the quality of treated drinking water due to low removals of these compounds.

3.2 Cocaine, BE and sucralose

Cocaine and its major metabolite BE were detected at all monitoring locations with both sampling techniques (Figure 1a). These compounds were detected at the two stations upstream of the WWTP discharge, which illustrates that the Grand River is impacted by discharges of CECs from several upstream WWTPs. A decrease in the level of cocaine was observed between the

two upstream sites. Since there was not a significant decline in the concentrations of sucralose at these two upstream locations (Figure 1b), the loss of cocaine may be explained by microbial or photolytic degradation of cocaine to transformation products [15, 33]. The ratios of BE to cocaine were approximately 1 at the two upstream locations.

The ratios of BE to cocaine in surface water were between 2.6 and 4.2 downstream of the WWTP discharge (Figure 2), which is within the range of values observed by others (Van Nuijs et al. 2009b). There was a trend to declining ratios of BE to cocaine with distance downstream in the river for data generated from both the grab samples and the POCIS (Figure 2). Since the ratios were similar for both POCIS and grab sample data, we can rule out that this trend was due to preferential adsorption of one of the two compounds onto the POCIS sorbent. These ratio data suggest that cocaine might be more persistent relative to BE. Since it has been shown that cocaine is more susceptible to microbial degradation than BE in aqueous matrices [6, 34], it may be that BE is lost through other fate processes, such as sorption to particulates, volatilization or photodegradation. For example Bijlsma et al. [9] showed that BE is removed in WWTPs slightly more efficiently than cocaine (90% for BE compared to 79% for cocaine); possibly through transport to sludge. Further studies are required to investigate the processes responsible for these changes in the ratios of cocaine and BE during wastewater treatment and in natural surface waters. The concentrations of BE and cocaine remained stable with distance downstream of the WWTP discharge (Figure 1a). This is not consistent with the data for sucralose, which showed a decline in concentrations between the first (561 ng L⁻¹) and second (260 ng L⁻¹) downstream sites, but then stable concentrations further downstream (Figure 1b).

The concentrations of cocaine and BE observed in all surface water samples were lower than the maximum concentrations reported in Europe. For instance, cocaine and BE were detected in surface water at concentrations between 10 and 111 ng L⁻¹ in Spain [35], at maximum concentrations of 115 and 520 ng L⁻¹, respectively in Belgium [36], and at maximum concentrations of 78 to 92 ng L⁻¹, respectively in south Wales [35-37]. The concentrations of sucralose in the Grand River (i.e. >100 ng L⁻¹) are consistent with previous studies [26, 28, 38].

Considering that the concentrations of cocaine and BE (4.2 and 10 ng L⁻¹, respectively) in the treated drinking water were not significantly different than the influent concentrations, removals in the DWTP could not be calculated. In contrast, a significant removal of 49% was found for sucralose in the DWTP (Table 1). A study conducted at a DWTP in Spain equipped with chlorination, ozonation and granular activated carbon filtration reported overall removal efficiencies for cocaine and BE of 100% and 89%, respectively, and BE was detected in the treated drinking water at mean concentrations of 45 ng L⁻¹, with a maximum of 130 ng L⁻¹ [35]. However, Boleda et al. [14] investigated tap water from DWTPs with different treatment technologies in several countries and found that BE and cocaine were present in treated drinking water at maximum concentrations of 15 and 2.9 ng L⁻¹, respectively and that the removal of these compounds is a strong function of the treatment technologies available. The DWTP monitored in the present study was operating at flows close to or greater than the maximum design capacity of the plant and it did not employ advanced treatment technologies, such as ozonation and activated carbon filtration. Therefore, it is not surprising that removals of sucralose, cocaine and BE were relatively low. In addition, van der Aa et al. [39] reported that only BE was detected in finished

drinking water in a study performed in the Netherlands, at a concentration below its limit of detection.

3.3 Amphetamines

Amphetamine, MDA and ephedrine were detected at concentrations >LOQ in surface water upstream (4 to 24 ng L⁻¹) and downstream (6 to 49 ng L⁻¹) of the WWTP discharge (Figure 1c). There is a trend of declining concentrations of ephedrine with distance downstream of the WWTP discharge (Figure 1c), indicating possible degradation of these compounds in surface waters or sorption to river sediments, considering that no such decline in concentration was observed for sucralose. The levels of ephedrine measured in the present study are in agreement with those reported by Valcárcel et al. [15], who detected ephedrine at concentrations between 1.76 and 14.70 ng L⁻¹ in a river in Spain. Postigo et al. [5] reported an ephedrine concentration of 145 ng L⁻¹ in another river in Spain. The higher levels of ephedrine in surface water, compared to other amphetamine stimulants are probably due to its widespread use as a decongestant and possibly in herbal medicines, high rate of excretion of the parent compound from the human body (22-99%) and incomplete removal during wastewater treatment [5]. Amphetamine was detected in surface waters at 6.8 and 3.38 ng L⁻¹ in Spain (Vazquez-Roig et al. 2010), but was not detected in a study in the USA [5, 40, 41], which is consistent with the data obtained in the present study. MDMA and methamphetamine were not detected in treated wastewater and in surface water in the present study, which may be due to low usage of Ecstasy as an illicit drug in this urban area.

For samples collected at the DWTP, amphetamine, methamphetamine and MDMA were not detected in any samples (untreated or treated). MDA and ephedrine were detected above their respective LOQs. Removals from drinking water during treatment could not be estimated because there was no significant difference in the concentrations in raw and treated drinking water. Few studies have investigated the presence of amphetamine stimulants in drinking water, but one study conducted in Europe showed that all amphetamines studied were completely removed during drinking water treatment [35].

3.4 Opioids

Methadone was not present at concentrations >LOQs in any samples and therefore, this compound was not included in Figure 1d. Heroin was not detected at concentrations above either its LOQ or LOD. As we previously did not detect heroin in 24-h composite samples from two WWTPs in Canada [22], it is likely that the results are not due to low uptake by the passive samplers, but rather due to a rapid rate of transformation. Low concentrations of this compound may be explained by its biotransformation to 6-acetylmorphine and morphine [42].

Fentanyl was detected in all samples (Figure 1d), despite its high excretion in humans as norfentanyl (26-55%) and its tendency to partition into the sludge phase [4, 43]. The low concentration of oxycodone and the high levels of fentanyl and ketamine in wastewater are significant from the perspective of drug abuse in the province of Ontario, Canada. The Oxycontin® formulation of oxycodone was banned in Ontario in 2012 because of concerns that this form of the drug, which can be easily crushed and dissolved in water for intravenous

injection, was contributing to high overdose deaths from abuse of this prescription pain killer [44]. Subsequent to this ban, drug enforcement agencies in Ontario reported an increase in the use of ketamine and fentanyl, and indeed there has been a rise in deaths as a result of fentanyl overdoses, as reported in 2012 by the Office of the Coroner for Ontario.

The concentrations of the opioid target compounds in surface water did not show any clear patterns between sampling points (Figure 1d). The concentrations of fentanyl, codeine and ketamine decreased downstream of the WWTP discharge, while the concentrations of morphine, acetylmorphine, acetylcodeine, dihydrocodeine, tramadol and oxycodone did not show any clear trends. These data may indicate that the latter compounds are not rapidly degraded in surface water. Of particular interest are the data for tramadol, which showed declining concentrations in surface water downstream of the WWTP discharge (Figure 1d). This was also observed in a study conducted in German surface waters where levels of tramadol were <25 to 381 ng L^{-1} and declined with distance from the WWTP discharge [45]. Indirect photodegradation has been shown to be the main process responsible for the removal of tramadol in surface water under ambient conditions [46]. EDDP was not detected in any surface water samples in the present study, although these compounds have been detected in river water samples in other studies [2, 15].

Several of the opioids were not detected, or were detected at concentrations below the LOQ in the untreated and treated drinking water. Removals could not be calculated for these compounds, including amphetamine, methamphetamine, MDMA, methadone and EDDP.

Several other opioids were detected in POCIS deployed in both untreated and treated drinking water, including fentanyl, ketamine, oxycodone, tramadol, morphine and codeine and dihydrocodeine. Using the POCIS data, removals could be calculated for compounds where there were statistically significant differences in the TWA concentrations in raw and treated drinking water; specifically for codeine (25%) and ketamine (49%). In studies conducted in Europe on opioids in drinking water, only methadone and EDDP were detected at concentrations >LOQs [2, 14]. The high number of opioid compounds detected in the current studies using POCIS demonstrates the value of using passive sampling techniques to monitor for trace quantities of CECs in drinking water.

4 CONCLUSIONS

The results of the present study demonstrate for the first time that drugs of abuse are present in Canadian surface waters impacted by wastewater discharges. The data also provide evidence that there is downstream transport of these compounds, such that wastewater discharges into surface waters have the potential to contaminate downstream sources of drinking water with drugs of abuse; albeit at relatively low concentrations.

Analysis of surface water samples indicated that the concentrations of cocaine, BE, morphine, acetylmorphine, dihydrocodeine and acetylcodeine and oxycodone did not decline in the Grand River with distance downstream from the WWTP discharge, which was consistent with the persistence of sucralose in the watershed. Overall, we accept our original hypothesis that

at least some of these DOAs are persistent in aquatic systems and surface water contamination associated with wastewater discharges may impact the quality of drinking water in urban watersheds.

5 SUPPLEMENTAL DATA

Figure S1. Comparison of estimated and measured amounts of the target compounds retained by the POCIS sorbent

Table S1. Chemical formulae, corresponding deuterated analogs, SPE recoveries, POCIS extraction efficiencies and POCIS sampling rates of target compounds

Table S2. Limits of detection (LODs) and limits of quantification (LOQs) in wastewater, surface water and drinking water

Table S3. Mean concentration ($n=3$, $\pm$ SD) in ngL^{-1} estimated from amounts accumulated in POCIS, or determined from analysis of grab samples collected at the time of deployment and retrieval of the POCIS, respectively.

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Figure 1. Average trends of concentrations of (a) cocaine and benzoylcegonine, (b) sucralose, (c) amphetamine-type stimulants and (d) other DOAs, in the Grand River, at the WWTP and DWTP (error bars represent one standard deviation). d-Codeine = dihydrocodeine. Heroin, acetylcodeine and acetylmorphine are not included since no sampling rate was available. Methadone is not included since it was not detected in any sample.

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571

572 **Figure 2.** Ratio of average benzoylecgonine (BE) concentration to average cocaine
573 concentration at a given sample location in POCIS and grab samples (each data point
574 represents the average concentration of n = 3 for POCIS and n = 6 for grab).

575

576 **Table 1.** Mean removal efficiencies of analytes at the DWTP (n=3)

Compounds	DWTP Removal Efficiencies
Cocaine & its metabolite	
Cocaine	-18
Benzoylcegonine	14
Sucralose	49*
Amphetamine-type stimulants	
Amphetamine	< LOQ
Methamphetamine	< LOQ
MDA	7
MDMA	< LOQ
Ephedrine	10
Opioids	
Codeine	25*
Acetylcodeine	NA
Dihydrocodeine	-10
Morphine	-2
Acetylmorphine	NA
Methadone	< LOQ
Heroin	NA
Tramadol	-39
Ketamine	49*
Oxycodone	-3
EDDP	< LOQ
Fentanyl	0

577 < LOQ Removal level not calculated due to concentrations < LOQ

578 NA Removal level not calculated due to unavailable sampling rates

579 * Statistically significant based on t-test (p<0.05)

580